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Detection of *BCL*-6 Rearrangements and *p53* Mutations in Malt-Lymphomas

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Twenty-seven lymphomas of mucosa-associated lymphoid tissue (MALT) derived from distinct anatomical sites were tested for the presence of genetic lesions commonly involved in B-cell lymphomagenesis, including activation of proto-oncogenes (*BCL*-1, *BCL*-2, *BCL*-6, and c-*MYC*), disruption of tumor suppressor loci (*p53,* 6q), and infection by viruses [Epstein-Barr virus (EBV), and Kaposi's sarcoma-herpesvirus/human herpesvirus-8 (KSHV/HHV-8)]. Sixteen low-grade and 11 high-grade MALT-lymphomas were included in the study. The presence of genetic lesions was tested by a combination of molecular approaches, including Southern blot hybridization, polymerase chain reaction (PCR), and PCR-single strand conformation polymorphism followed by DNA direct sequencing. Alterations of *BCL*-1, *BCL*-2, or c-*MYC,* as well as infection by KSHV/HHV-8, scored negative in all MALT-lymphomas analysed. Conversely, rearrangements of *BCL*-6 and mutations of *p53* clustered with a fraction of high-grade MALT-lymphomas. Deletions of 6q occurred in selected cases of both low- and high-grade MALT-lymphomas, whereas a monoclonal infection by EBV was restricted to one single patient. These data corroborate the notion that the molecular pathogenesis of MALT-lymphomas differs substantially from that of nodal B-cell lymphomas. Occasionally, however, a proportion of high-grade MALT-lymphomas may harbor selected genetic lesions among the ones commonly involved in nodal B-cell lymphomagenesis. Am. J. Hematol. 56:206–213, 1997.

Key words: lymphoma; mucosa-associated lymphoid tissue; oncogene; tumor suppressor gene

INTRODUCTION

Lymphomas of mucosa-associated lymphoid tissue (MALT) represent a distinct subgroup of B-cell non-Hodgkin's lymphomas (NHL) arising in extranodal sites, most commonly the gastrointestinal tract, thyroid, testis, lung, and salivary glands among others [1,2]. Low-grade MALT-lymphomas are constituted by ''centrocyte-like'' small cells and frequently display characteristic lymphoepithelial lesions [1–3]. Low-grade MALT-lymphomas may undergo histologic transformation to a high-grade pattern with variable proportions of residual small cell

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foci comingled with the emerging large cell population [1–3].

The pathogenesis of MALT-lymphomas has been elucidated to a certain extent. In the case of gastric MALT-lymphomas, the consistent association with infection by *Helicobacter pylori* (HP) as well as the clinical evidence of lymphoma regression after HP eradication have suggested that HP has a role in causing these neoplasms [4–6]. In addition to HP infection, chronic antigen stimulation, possibly involving self-antigens, has also been implicated in the pathogenesis of gastric MALT-lymphomas [7,8].

In contrast to nodal B-cell NHL, which tend to systematically associate with distinct genetic lesions [9], no cancer-related gene has been found to be consistently involved in MALT-lymphomas. Some of the genetic lesions occurring in nodal B-cell NHL, namely rearrangements of *BCL*-1, *BCL*-2, and c-*MYC*, are absent in MALT-lymphomas [10–13], whereas other, such as rearrangements of *BCL*-6, deletions of 6q, and infection by Kaposi's sarcoma-herpesvirus/human herpesvirus-8 (KSHV/HHV-8), have not been investigated extensively. Finally, the tumor progression from low- to high-grade MALT-lymphomas appears to associate with genetic alterations at the *p53* locus in some cases [14].

In this study, we have tested a panel of low- and high-grade MALT-lymphomas arising in distinct anatomical sites for the presence of a number of genetic lesions relevant to B-cell lymphomagenesis, including activation of proto-oncogenes, disruption of tumor suppressor loci, and viral infection. Overall, our data corroborate the notion that the molecular pathogenesis of MALT-lymphomas is distinct from that of nodal B-cell NHL. Notably, however, selected cases of MALT-lymphomas may occasionally harbor genetic lesions shared also by nodal B-cell NHL, including rearrangements of *BCL*-6, mutations of *p53*, and deletions of 6q.

MATERIALS AND METHODS

Pathologic Samples and DNA Extraction

Biopsy samples of involved organs from 27 patients with MALT-lymphomas were collected during the course of standard diagnostic procedures. All bioptic specimens were collected at diagnosis, prior to therapy for the lymphoproliferative disease. Diagnosis was based on analysis of histopathology, immunophenotypic analysis of cell surface markers, and immunogenotypic analysis of immunoglobulin (Ig) gene rearrangement, as previously reported [1,2]. Low-grade MALT-lymphomas were pathologically defined based on previously reported morphologic criteria [1–3]. High-grade MALT-lymphomas were pathologically defined based on the size of the predominant cell population and the presence of a diffuse architecture. Notably, with respect to MALT-lymphoma cases classified as high grade, our study included only samples in which one or more foci of a low-grade MALT-lymphoma component could be specifically identified [1,2,14]. On these grounds, 16 cases were classified as low-grade MALT-lymphomas and 11 cases as high-grade MALT-lymphomas. The site of the MALT-lymphoma was the stomach in 19 cases, the gut in 2 cases, the thyroid in 3 cases, and the parotid gland, the lung, and the testis in 1 case each. In most samples, the fraction of the malignant cells in the pathologic specimen was greater than 50% (and in all cases greater than 30%), as determined by tissue section immunohistochemical analysis of cell surface markers and by Ig gene rearrangement analysis. DNA was purified by digestion with proteinase K, ''salting out'' extraction, and precipitation by ethanol [15].

Southern Blot Analysis

For Southern blot analysis [16], 6 to 10 μg of genomic DNA was digested with the appropriate restriction enzyme, electrophoresed in a 0.8–1% agarose gel, denatured, neutralized, transferred to Hybond C^+ filters (Amersham, Amersham Place, UK), and hybridized to probes that had been ^{32}P-labeled by the random priming extension method [17]. Filters were washed in 0.2 × SSC (NaCl/Na citrate/0.5% sodium dodecyl sulfate) for 2 hr at 60°C and then autoradiographed using intensifying screens.

DNA Probes and Experimental Strategy

Ig gene rearrangement analysis was performed using a J_H probe on *Hind*III, *Eco*RI, and *Bam*HI digests and a J_k probe on *Bam*HI digests [18,19].

The configuration of the *BCL*-6 locus was investigated by Southern blot analysis using a probe (Sac 4.0; kind gift of Dr. R. Dalla-Favera) and restriction enzymes (*Bam*HI and *Xba*I) that, in combination, explore a region of 15.2 Kb containing the 5′ portion of the *BCL*-6 gene [20,21]. This same region was previously shown to contain the cluster of chromosomal breakpoints detected in nodal NHL [20]. Cases showing an abnormally migrating band in only one digest were further studied by hybridizing *Bam*HI and *Xba*I digests to a probe (Sac0.8; kind gift of Dr. R Dalla-Favera) derived from the *BCL*-6 first intron, which, being located 3′ of the breakpoint cluster, explores the reciprocal chromosome 3 [22]. Only cases showing abnormally migrating bands with two restriction enzymes and/or two probes were scored as rearranged.

The organization of the c-*MYC* locus was analyzed by hybridization of *Eco*RI and *Hind*III digested DNA to the human c-*MYC* probe MC413RC, representative of the third exon of the c-*MYC* gene (a kind gift of Dr. R Dalla-Favera) [23].

The configuration of the *BCL*-2 locus was investigated by the use of probes pFL-1 and pFL-2 on *Bam*HI digests

and pB16 on *Hind*III digests [24,25]. The configuration of the *BCL*-1 locus was tested by applying probes MTC (probe ''b'') and p94PS on *Bcl*I and *Eco*RI digests, respectively [26,27].

The presence of the Epstein-Barr virus (EBV) genome was investigated with a probe specific for the EBV genomic termini (5.2 Kb *Bam*HI-*Eco*RI fragment isolated from the fused *Bam*HI terminal fragment NJ-het), which allows the definition of clonality of the EBV infection [28].

The analysis of chromosomal deletions at 6q27 and 17p13 was performed as previously reported [29,30]. Briefly, by using two highly polymorphic probes (heterozygosity > 95%) mapping within the chromosomal region of interest, the presence of a deletion can be rapidly tested in the absence of paired normal DNA [29,30]. Thus, the presence of 6q deletions was studied by using two highly polymorphic cosmid clones mapping to 6q27: CEB3/D6S132 and CEB4/D6S133 on *Hind*III digests and *Eco*RI digests, respectively [31]. Deletions were scored when cases displayed only one band (i.e., one allele) with both probes or, alternatively, when they displayed a difference in signal intensity between the two alleles recognized by each probe in both CEB3/D6S132 and CEB4/D6S133 hybridizations. The presence of deletions at 17p13, the site of the *p53* tumor suppressor locus, was investigated by using two highly polymorphic probes mapping to this chromosomal region (pYNZ22.1/D17S30 and p144D6/D17S34; purchased from ATCC, Rockville, MD) on *Hinf*I digests. Criteria for scoring deletions at 17p13 were as reported above for deletions of 6q.

Oligonucleotides

All the oligonucleotides utilized in this study have been synthesized by the solid-phase triester method.

Polymerase Chain Reaction (PCR) Analysis of *BCL*-2 Rearrangements

PCR analysis of rearrangements of the *BCL*-2 gene with an Ig_H locus was performed on tumor genomic DNA following a previously reported method [32]. For each tumor sample, distinct PCR reactions were performed for the analysis of the major breakpoint region (MBR) and minor cluster region (mcr) of the *BCL*-2 gene.

Analysis of KSHV/HHV-8 DNA Sequences

The presence of KSHV/HHV-8 DNA sequences was tested on tumor genomic DNA using a previously reported assay [33]. Briefly, PCR was performed with oligonucleotides $KS330_{233}$-F (5′-AGCCGAAAGGATTCCACCAT-3′) and $KS330_{233}$-R (5′-TCCGTGTTGTCTACGTCCAG-3′), which amplify a 233 bp fragment from the KS330Bam region of KSHV/HHV-8. Upon electrophoretic migration, the PCR product was hybridized with a radiolabelled oligonucleotide probe located within the amplified gene fragment ($KS330_{233}$I: 5′-TGCAGCAGCTGTTGGTGTACCACAT-3′) [33]. Control amplification was performed by using primer sets derived from the K-*RAS* genomic sequence [34] and/or the *p53* sequence (see below).

p53 Polymerase Chain Reaction: Single Strand Conformation Polymorphism (PCR-SSCP) and PCR Product Restriction Analysis

PCR-SSCP analysis was modified from Orita et al. [35]. Briefly, PCR was performed with 100 ng of genomic DNA, 10 pmol of each primer (names and sequence of *p53* primers used in this study have been reported elsewhere) [36], 2.5 μM dNTPs, 1 μCi of [α-^{32}P]dCTP (Amersham; specific activity, 3,000 Ci/mmol; 1 Ci = 37 GBq), 10 mM Tris-HCl (pH 8.8), 50 mM KCL, 1 mM $MgCl_2$, 0.01% gelatin, 0.5 U AmpliTaq polymerase (Perkin-Elmer, Norwalk, CT) in a final volume of 10 μl. Thirty cycles of denaturation (94°C), annealing (annealing temperatures were optimized for each pair of primers), and extension (72°C) were performed in a temperature controller (DNA Thermal Cycler, Perkin-Elmer, Norwalk, CT). The reaction (2 μl) was diluted 1:25 in 0.1% $NaDodSO_4$ (SDS), 10 mM EDTA, and further mixed 1:1 with sequencing stop solution [16]. Samples were heated at 95°C for 5 min, chilled on ice, and immediately loaded (3 μl) onto a 6% acrylamide-TBE gel containing 10% glycerol. Gels were run at 8 W for 12 to 15 hr at room temperature, fixed in 10% acetic acid, air dried, and analyzed by autoradiography using an intensifying screen for 6 to 72 hr. At least three positive controls, represented by tumor cases carrying known *p53* mutations [36], were included for each PCR fragment tested. For cases showing an abnormal PCR-SSCP migration pattern in *p53* exon 6, the presence of the *Taq*I population polymorphism was tested as previously reported [37].

Direct Sequencing of DNA PCR Products

PCR was performed with 500 ng of genomic DNA, 20 pmol of each primer, 200 μM dNTPs, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1 mM $MgCl_2$, 0.01% gelatin, and 2.5 U *Taq* polymerase. The number and conditions of amplification cycles were as described above. PCR products were purified using a QIAGEN (GmbH, Germany) DNA extraction kit according to the manufacturer's instructions. For DNA direct sequencing [36], approximately 0.5 pmol of purified DNA PCR product was denatured at 95°C for 5 min and annealed in the presence of 10 pmol of primer at room temperature for 1 min. Sequencing reactions were performed with reagents supplied with the Sequenase version 2.0 sequencing kit (USB, Cleveland, OH) following the manufacturer's

Table I. Distribution of Genetic Lesions in MALT-Lymphomas*

Histology[a]	*BCL*-1	*BCL*-2	*BCL*-6	c-*MYC*	*p53*	17p$^-$	6q$^-$	EBV	HHV-8
Low-grade	0/16	0/16	0/16	0/16	0/16	0/16	2/16	0/16	0/16
High-grade	0/11	0/11	3/11	0/11	2/11	2/11	2/11	1/11	0/11

*Low-grade and high-grade MALT-lymphomas were classified based on previously reported morphologic criteria [1–3].

specifications. [^{35}S]-dATP was included in the sequencing mixture. Both strands were sequenced for each DNA fragment analyzed.

RESULTS

Clonal Analysis

All samples were tested for Ig rearrangements using a J_H and a J_κ probe on multiple restriction enzyme digestions. A major band of Ig rearrangement was detectable in all specimens, confirming their monoclonal B-cell origin (data not shown).

Analysis of Proto-Oncogenes

Alterations of the following proto-oncogenes were investigated in MALT-lymphomas: *BCL*-1, *BCL*-2, *BCL*-6, and c-*MYC*. No rearrangements of *BCL*-1, *BCL*-2, or c-*MYC* were detected in any of the MALT-lymphoma samples tested (data not shown). Conversely, rearrangements of the *BCL*-6 gene were detected in 3/27 (11.1%) MALT-lymphomas (Table I and Fig. 1). The distribution of *BCL*-6 alterations among the histologic variants of MALT-lymphomas was not random, since rearrangements of *BCL*-6 were restricted to cases displaying a high-grade histology (3/11; 27.2%), whereas they were consistently absent in low-grade MALT-lymphomas (0/16; 0%). The three cases carrying *BCL*-6 rearrangements originated from distinct anatomic sites, including the thyroid (case 166), the stomach (case 133), and the testis (case 135). In all three cases, the rearrangement of *BCL*-6 was the sole genetic lesion detectable among the ones investigated. The pattern and genomic location of the *BCL*-6 breakpoints observed in MALT-lymphomas was similar to that most commonly found in nodal B-cell NHL [21,38,39].

Analysis of Tumor Suppressor Loci

The analysis of tumor suppressor loci in MALT-lymphomas included the study of *p53* mutations as well as the investigation of chromosomal deletions at 17p13 and 6q27. 17p13 is the mapping site of *p53* [29], whereas 6q27 represents the region of minimal deletion involved by 6q abnormalities in 30% of nodal B-cell NHL [40].

Mutations of *p53* were detected in 2/11 (18.1%) cases of high-grade MALT-lymphomas, whereas they were negative in all cases with a low-grade histology (Table I and Fig. 2). The two cases carrying *p53* mutations (cases

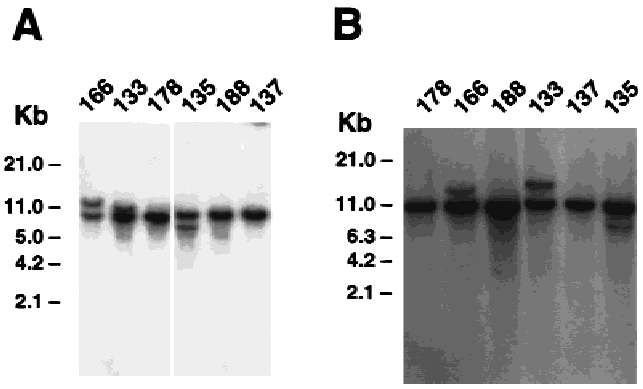

Fig. 1. Southern blot analysis of *BCL*-6 rearrangements in representative cases of MALT-lymphomas. Genomic DNAs were digested with *Bam*HI (A) and *Xba*I (B), electrophoresed in a 0.8% agarose gel, transferred to a nitrocellulose filter and hybridized to a *BCL*6 probe (Sac 4.0) labeled by the random priming extension method. A *BCL*-6 rearrangement was observed in MALT-lymphoma cases 133, 135, and 166.

129 and 130) were MALT-lymphomas of the stomach. No other genetic lesion was detected in these two samples. Case 129 carried a CAC→TAC transition at codon 296, leading to a Hys→Tyr aminoacid substitution. Case 130 carried a CAT→CGT transition at codon 179, leading to a Hys→Arg aminoacid substitution. The mutation of case 130 was hemizygous, as demonstrated by DNA direct sequencing and loss of heterozygosity analysis by Southern blot of the 17p13 region (results of Southern blot analysis are shown in Fig. 2D and E), whereas the mutation of case 129 was heterozygous. In addition to case 130, deletion at 17p13 was also detected in a high-grade MALT-lymphoma of the stomach (case 173) displaying only wild type *p53* sequences (not shown). No other genetic alterations were detected in this case.

Based on the criteria adopted for scoring 6q deletions with the CEB3/D6S132 and CEB4/D6S133 probes (see Materials and Methods), deletions at 6q27 were scored in 4/27 MALT-lymphomas (14.8%), including 2/16 (12.5%) low-grade and 2/11 (18.1%) high-grade cases (Table I and Fig. 3). Two patients with a 6q deletion had a MALT-lymphoma of the stomach (cases 157 and 141) whereas the other two had a MALT-lymphoma of the thyroid (cases 159 and 183). In three patients, the 6q deletion was the sole genetic lesion among the ones tested, whereas in the fourth case (case 159) EBV infec-

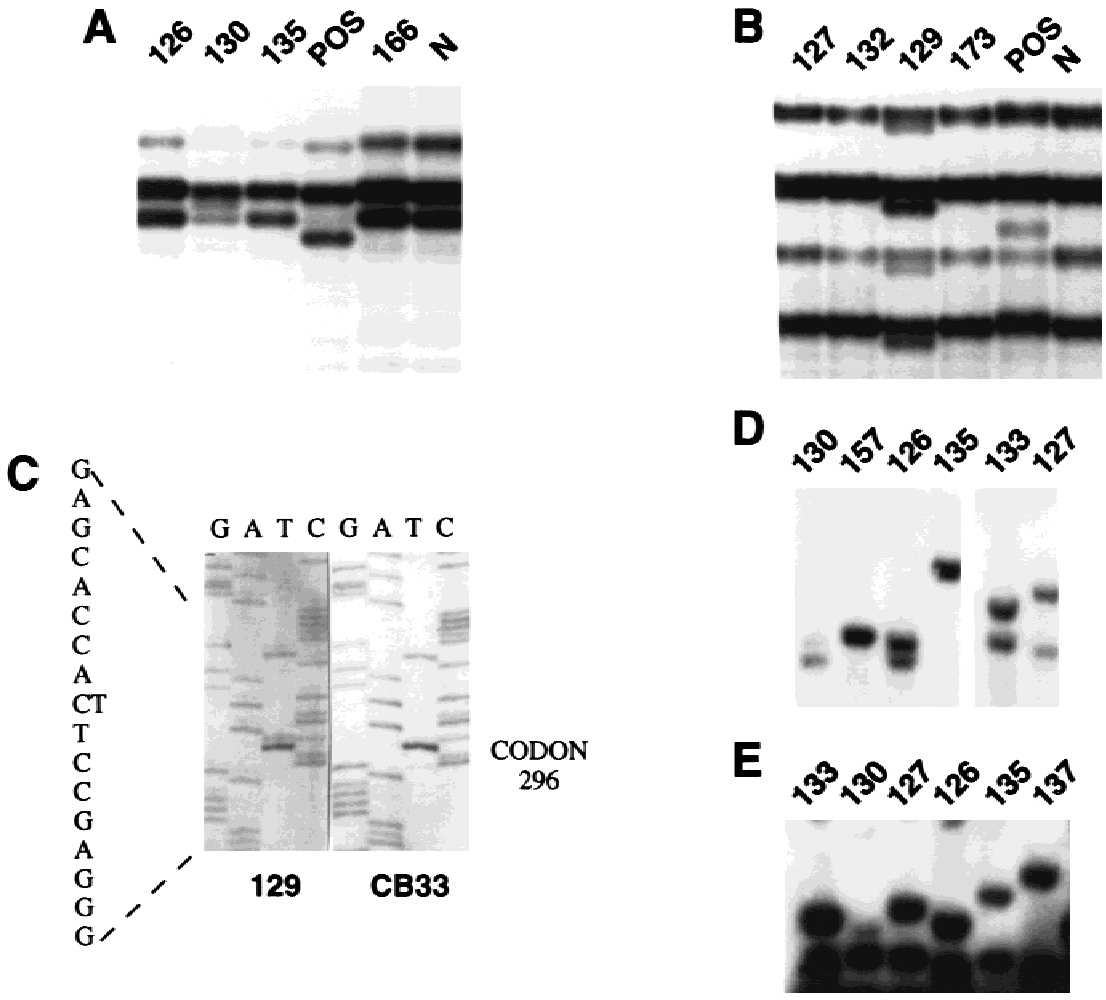

Fig. 2. Analysis of the *p53* locus by PCR-SSCP (A and B), DNA PCR-direct sequencing (C), and Southern blot studies (D and E) in cases of MALT-lymphomas. PCR-SSCP analysis of *p53* exon 5 (A) and exon 8 (B) are shown. Samples were scored positive when their migration pattern differed from the normal control (N). Positive controls (POS) were included for each exon. An abnormal migration pattern was detected in MALT-lymphoma cases 130 (exon 5) and 129 (exon 8). C: Analysis by DNA direct sequencing of the *p53* gene in case 129 scoredpositive by PCR-SSCP. The coding strands of case 129 (mutated at codon 296) and of CB33, a B-lymphoblastoid cell line harboring a germline *p53* gene used as a normal control, are shown. D and E: Allelic representation of the 17p13 locus in MALT-NHL by Southern blot hybridization with the pYNZ22.1 (D) and p144D6 (E) probes. Cases were scored positive for LOH when displaying only one allelic band or allelic bands of unequal intensities with both probes (see text for details). Among the cases shown, a 17p13 deletion was scored in case 130.

tion of the tumor clone was present in combination with the 6q27 deletion (see below). One additional case (case 186) displayed a difference in the reciprocal intensity of the allelic bands recognized by CEB4/D6S133 but not of those recognized by CEB3/D6S132 (Fig. 3). Thus, this case did not match the criteria adopted for scoring 6q deletions (see Materials and Methods). Hypothetically, case 186 harbored a limited deletion of 6q, which spanned the CEB4/D6S133 locus but not the CEB3/D6S132 locus.

Analysis of Viral Sequences

The presence of EBV and KSHV/HHV-8 sequences was tested by Southern blot and by PCR, respectively. A monoclonal pattern of EBV infection was detected in one case of high-grade MALT-lymphoma of the thyroid (case 159) (Table I and Fig. 4). This same case also carried a deletion of 6q. The monoclonality of EBV infection in case 159 suggests that viral infection was already present in the neoplastic cells before tumor expansion. All other samples of MALT-lymphomas were negative for EBV infection (Table I). All MALT-lymphomas were also devoid of KSHV/HHV-8 DNA sequences as defined by PCR analysis of the viral DNA (Table I). The presence of a PCR inhibitor or DNA degradation in samples negative for KSHV/HHV-8 sequences could be excluded, since all cases could be amplified with K-*RAS* and/or *p53* primers.

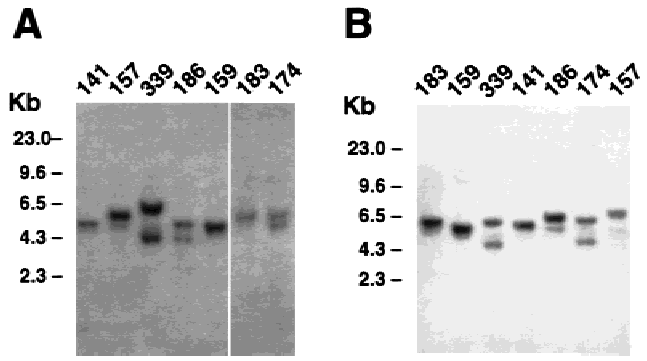

Fig. 3. Loss of heterozygosity analysis at 6q27 in cases of MALT-lymphomas. Genomic DNAs were digested with *Hind*III (A) or *Eco*RI (B) and subjected to Southern blot hybridization using two highly polymorphic probes mapping to 6q27, i.e., CEB3/D6S132 (A) and CEB4/D6S133 (B). Cases were scored positive for LOH when displaying only one allelic band or allelic bands of unequal intensities with both probes (see text for details). Among the MALT-lymphomas shown, cases 141, 157, 159, and 183 were scored positive for LOH at 6q27.

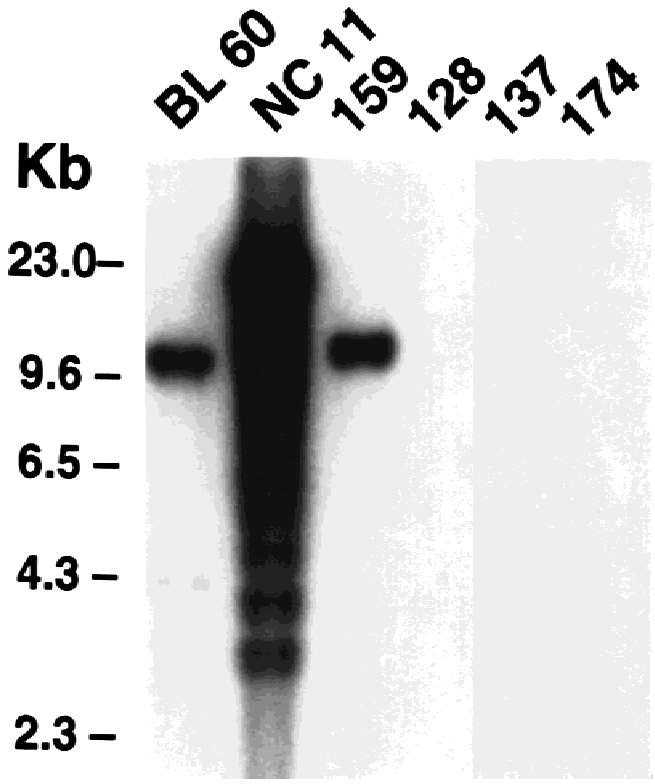

Fig. 4. Southern blot analysis of EBV infection in MALT-lymphomas. EBV infection was tested by Southern blot analysis on *Bam*HI digests using a probe representative of the EBV genomic termini allowing the definition of the clonality of EBV infection. BL60 (a Burkitt's lymphoma cell line) and NC11 (a B-lymphoblastoid cell line) were used as controls for monoclonal and polyclonal EBV infection, respectively. A pattern of monoclonal EBV infection was observed in case 159, representing a MALT-lymphoma of the thyroid, whereas cases 128, 137, and 174 scored negative for EBV sequences.

DISCUSSION

In contrast to nodal B-cell NHL, no cancer-related gene has been found to specifically and consistently associate with MALT-lymphomas [10–13]. Recently, Wotherspoon et al. have reported on the recurrence of trisomy 3 among MALT lymphomas [41]. The high frequency of this cytogenetic abnormality in these tumors suggests that it may harbor a pathogenetic significance, although the identification of the relevant gene is presently lacking [41].

This study investigated a panel of low- and high-grade MALT-lymphomas arising from distinct mucosal sites for the presence of genetic lesions known to be involved in B-cell transformation and tumorigenesis. With respect to rearrangements of *BCL*-1, *BCL*-2, and c-*MYC*, our data confirm previous evidence indicating that these loci are not involved in the pathogenesis of low-grade MALT-lymphomas nor in their transformation to high-grade lesions [10–13]. Similarly, MALT-lymphomas consistently lack KSHV/HHV-8 infection, which is frequently detected in a peculiar type of B-cell NHL termed body-cavity-based lymphomas [42].

Two distinct genetic lesions commonly associated with B-cell lymphomagenesis, namely rearrangements of *BCL*-6 and mutations of *p53*, are detected at a certain frequency in MALT-lymphomas (Table I). The involvement of *BCL*-6 in MALT-lymphomas is intriguing since *BCL*-6 maps to 3q27 [20], a chromosomal site that is triplicated in a large fraction of MALT-lymphomas [41]. The lack of cytogenetic analysis in the present study prevents any direct correlation between the presence of a *BCL*-6 rearrangement and trisomy 3. Our data, however, contrast the hypothesis that *BCL*-6 might be the gene relevant to trisomy 3 in MALT-lymphomas. Indeed, *BCL*-6 rearrangements are consistently absent in low-grade MALT-lymphomas, which conversely display trisomy 3 in more than 50% of the cases [41]. It is thus likely that trisomy 3 in MALT-lymphomas involves presently unidentified loci.

Regarding *p53* inactivation, point mutations of *p53* occur in a fraction of high-grade MALT lymphomas (Table I). In contrast to Du et al. [14], no *p53* alterations were observed among low-grade cases in our study. This discrepancy may stem from several factors, including the technical approach used for the detection of *p53* mutations or differences in the size of the tumor panel tested. The possibility that our failure to detect *p53* mutations in low-grade MALT-lymphomas was due to the limited sensitivity of PCR-SSCP could be ruled out, since only cases containing $\geqslant$30% (and in most cases $\geqslant$50%) tumor cells were included in our study whereas PCR-SSCP allows the detection of a mutation present in <10% of cells

[36]. Rather, the use of tissue microdissection for the isolation of lymphomatous cells, as performed in the study of Du et al. [14], may provide an attractive explanation for the discrepancy between the two studies. Whereas the whole tumor population scores negative for *p53* mutations (this report), microdissected tissue fragments displaying *p53* alterations may putatively represent small foci of apparently indolent cells constituting precursor lesions of high-grade MALT-lymphomas [14]. Future studies are needed in order to validate this hypothesis.

Deletions of 6q and EBV infection were also detected in a restricted number of MALT-lymphomas (Table I). The detection of 6q deletions in MALT-lymphomas expands the spectrum of B-cell disorders associated with this abnormality [40]. As in B-cell nodal NHL, 6q deletions among MALT-lymphomas occur both in low- and high-grade tumors. Infection by EBV has been sporadically reported in association with MALT-lymphomas [13,43]. In our series, only one case, represented by a thyroid MALT-lymphoma, carried EBV DNA sequences. Notably, the pattern of EBV infection of the tumor was monoclonal, indicating that infection had occurred prior to clonal expansion. This suggests that, as also assumed for other EBV-infected B-cell neoplasms [44], EBV infection may contribute to MALT-lymphomagenesis at least in rare instances.

Overall, our data confirm the notion that the molecular pathogenesis of both low-grade and high-grade MALT-lymphomas significantly differs from that of nodal B-cell NHL [1,2,9]. In particular, when considering high-grade MALT-lymphomas, the frequency of *BCL*-6 rearrangements in these tumors is markedly lower than that detected in nodal B-cell diffuse large cell lymphomas [21,38,39]. Also, high-grade MALT-lymphomas are consistently devoid of *BCL*-2 lesions, which conversely occur in a substantial fraction of nodal B-cell diffuse large cell lymphomas [45]. Finally, it is noteworthy that the majority of high-grade MALT-lymphomas harbor at least one genetic lesion among the ones presently known to be involved in B-cell lymphomagenesis. In this study, 7/11 (63.6%) high-grade MALT-lymphomas carried alterations of either *BCL*-6, *p53*, or 6q. Whether these genetic lesions are acquired during progression from low-grade MALT-lymphoma to a high-grade histology, or whether they define a peculiar subset of MALT-lymphomas presenting at diagnosis with a high-grade component, is presently unknown.

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